

SERIES 50 CHAMBERS

Series 50 chambers from Warner Instruments are designed for transepithelial measurements in a small footprint. These temperature controllable chambers can be easily mounted onto a microscope and can be used in either an open or closed bath configuration.

THE RC-50

The **RC-50** is a low profile, horizontally mounted Ussing chamber incorporating special features for confocal imaging. This chamber is designed to be used with both inverted and upright microscopes, and supports both open or closed bath modes.



In the closed bath configuration, a #1 glass coverslip forms both the top and bottom of the chamber allowing it to be used with both inverted and upright microscopes. The chamber platform (basolateral side) is beveled to permit maximum access by the microscope objective to the bottom coverslip. The basolateral viewing aperture is 17.7 mm in diameter and the apical viewing aperture is 22.8 mm. Resistive heating elements are integral to the chamber platform and are compatible with Warner's **TC-324B** and **TC-344B** Heater Controllers.

The **RC-50** comes complete with:

- Chamber
- Open bath cover
- Platform
- Basolateral chamber coverslip mounting tool
- 25 mm round glass coverslips (#1, 100 ea.)
- 22 x 40 mm glass coverslips (#1, 50 ea.)
- Coverslip removal tools (2 ea.)
- 23 gauge needles (4 ea.)
- Aspirator tubes (1 ea. large and small)
- PE-90 tubing (10 ft.)
- PE-160 tubing (10 ft.)
- Perfusion tube cleanout tool

THE RC-50E

The **RC-50E** carries all the same features as the **RC-50** with the addition of incorporated current and voltage electrodes into the design. An electrode kit comprised of a set of Ag^+ voltage and current electrodes, and electrode housings is provided. Voltage electrode housings are constructed from disposable pipette tips and are easily replaced.

The **RC-50E** includes all of the above along with 2 each of the following:

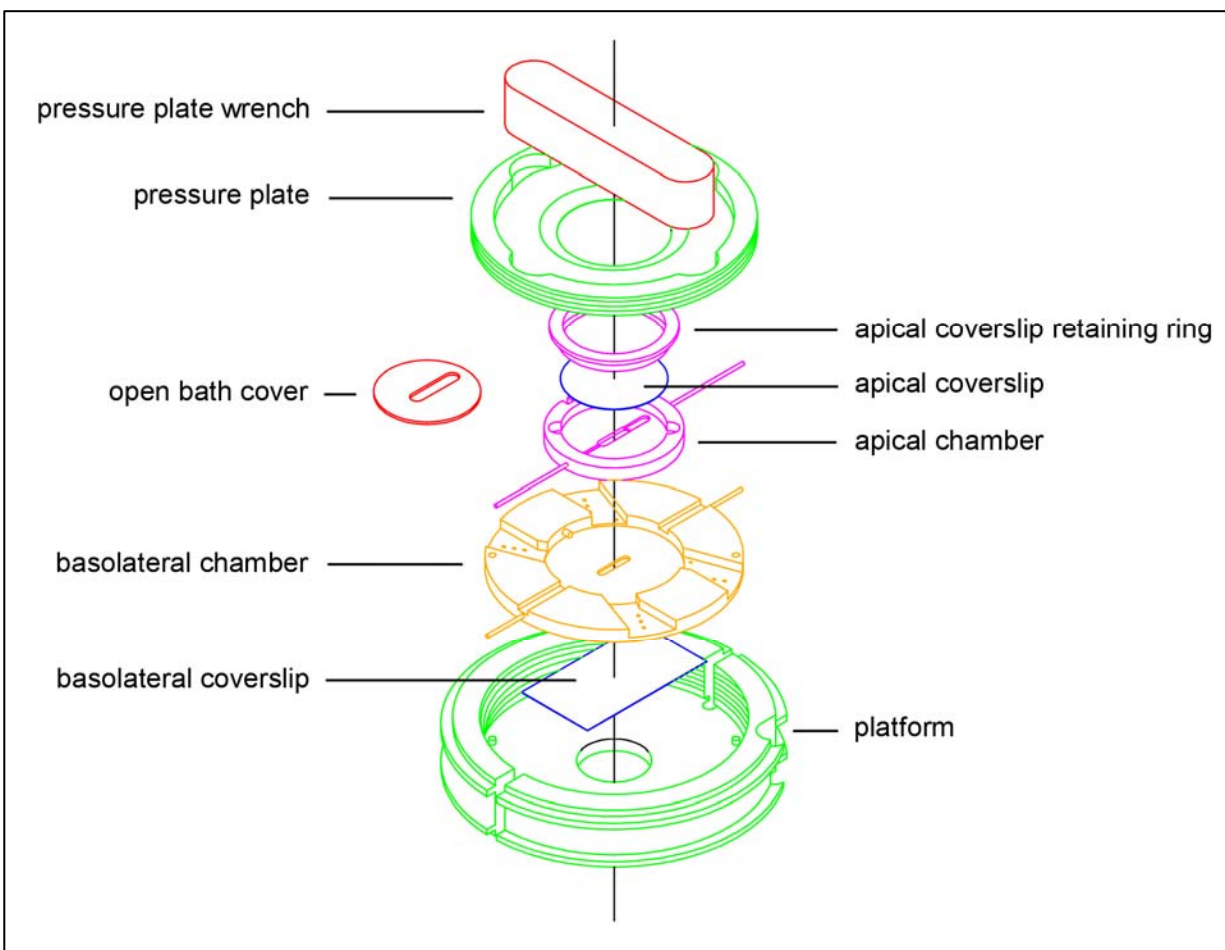
- Ag^+ wire voltage electrodes
- Ag^+ wire current electrodes
- Current electrode T-fittings
- Current electrode electrode-housings
- Voltage electrode electrode-housings

NOTE: General instructions applying equally to both chambers will refer to the model number as **RC-50/E**.

ASSEMBLY

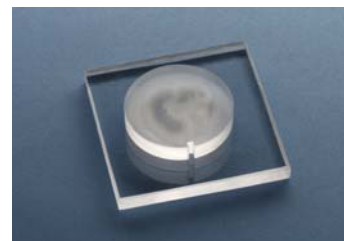
The general procedure for assembling **RC-50/E** chambers is to first attach the BASOLATERAL COVERSLIP to the BASOLATERAL CHAMBER. This is followed by submerging the BASOLATERAL CHAMBER and stretching the epithelia. Next, the APICAL CHAMBER is assembled, mounted, and sealed. Finally, solution delivery tubing is positioned and the chamber is placed into the PLATFORM. If using the **RC-50E**, electrodes are formed and inserted into their respective ports.

At this point you are ready to mount the **RC-50/E** onto your microscope, usually via a stage adapter.



Assemble the Basolateral Chamber

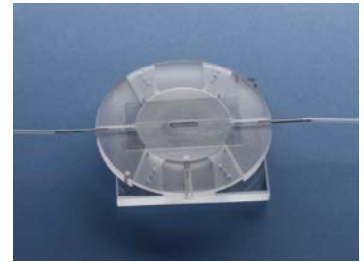
1. Begin by locating the BASOLATERAL CHAMBER COVERSLIP MOUNTING TOOL (shown to the right).
2. Place the BASOLATERAL CHAMBER upside down onto the MOUNTING TOOL. Note that the tool is 'keyed' to accept the associated pin in the BASOLATERAL CHAMBER.
3. Locate the 22x40 mm indentation on the underside of the BASOLATERAL



CHAMBER. Using a small paintbrush (size 0 or 00 works well), apply a thin layer of vacuum grease to this indentation.

NOTE: Avoid getting grease into the perfusion ports or flow channels which run from the ports to the oval aperture.

4. Press a clean 22x40 mm coverslip into the indentation to evenly distribute the grease. Remove the coverslip and dispose. Clean any excess grease from around the aperture and flow channels, and secure another clean 22x40 mm coverslip into place. A properly mounted BASOLATERAL COVERSIP is shown to the right.
5. Remove the chamber from the BASOLATERAL CHAMBER COVERSIP MOUNTING TOOL and place it upright on a clean surface. Using the paintbrush, apply a thin coating of vacuum grease to the circular area surrounding the oval aperture in the bottom of the BASOLATERAL CHAMBER.

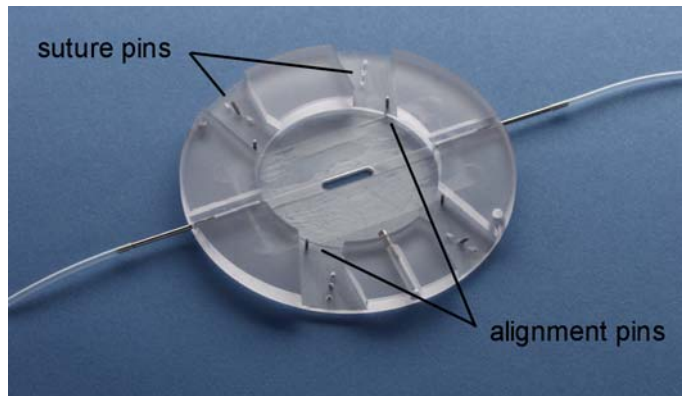


NOTE: Avoid getting vacuum grease into the oval aperture or you will need to clean and re-assemble the basolateral chamber.

Mount the Tissue

6. Submerge the assembled BASOLATERAL CHAMBER into a previously prepared 6" Petri dish which has been bottom-coated with a layer (~ 1/4") of Sylgard. Use a sufficient quantity of working buffer to completely submerge the chamber.
7. Place your prepared tissue over the aperture in the BASOLATERAL CHAMBER and stretch it into position using suture lines.

NOTE: Slots have been milled into the basolateral chamber to facilitate placement and stretching of the tissue. Suture pins are provided to act as anchor points for the suture lines. If desired, the alignment pins can be used to provide a clean 90° angle between the suture lines.



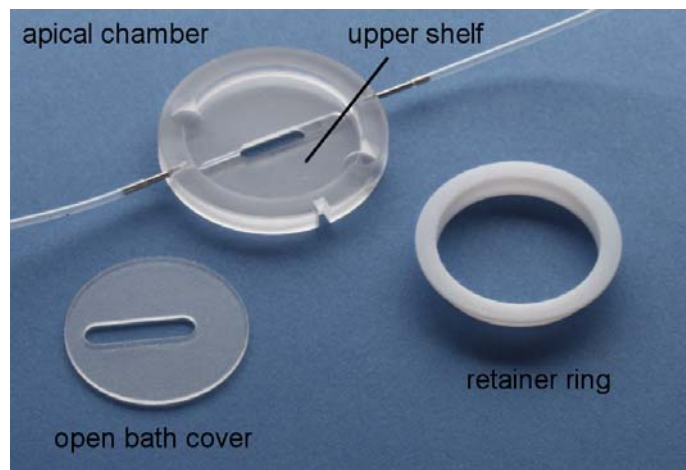
Prepare the Apical Chamber for Assembly

8. Remove the RETAINER RING and coverslip (or OPEN BATH COVER) from the APICAL CHAMBER (see image below).
9. Flip the APICAL CHAMBER over and apply a thin film of vacuum grease to flat underside of the chamber.

NOTE: Avoid getting vacuum grease into the oval aperture.

Form the Ussing Chamber

10. Retrieve the BASOLATERAL CHAMBER from the petri dish, dry the underside, and place it onto a clean surface.



11. Orient the APICAL CHAMBER to the BASOLATERAL CHAMBER by aligning the perfusion ports between the two sections.

NOTE: A guide pin in the basolateral chamber is provided to assure proper orientation between the basolateral and apical chambers.

12. Form the Ussing Chamber by gently pressing the APICAL CHAMBER onto the tissue spanning the aperture in the BASOLATERAL CHAMBER. Use only enough force to provide a secure seal between the two chamber halves.

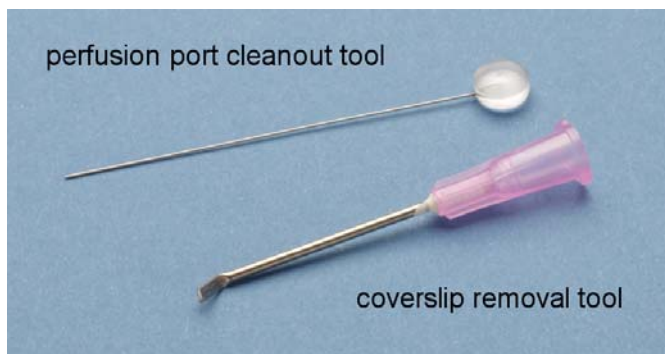
Seal the Ussing Chamber

13. Using the paintbrush, apply a thin layer of vacuum grease to the upper shelf on the APICAL CHAMBER.

NOTE: Avoid getting grease into the perfusion ports or flow channels which run from the ports to the oval aperture.

14. Press a clean 25 mm round coverslip into the indentation to evenly distribute the grease. Remove the coverslip and dispose. Carefully clean any excess grease from around the aperture and flow channels.

NOTE: A COVERSIP REMOVAL TOOL has been provided to aid in removal of coverslips. If the perfusion ports become clogged, clear them with the PERFUSION PORT CLEANOUT TOOL. (See **Maintenance**).



15. If configuring the **RC-50/E** for *closed bath* use, then place a clean 25 mm coverslip onto the upper shelf and secure using the RETAINER RING.
16. If configuring the **RC-50/E** for *open bath* use, then replace the coverslip in step 15 with the OPEN BATH COVER (see page 3).

NOTE: Be sure to align the asymmetric geometry of the open bath cover to the associated cutout on the apical chamber upper shelf.

Attach Perfusion lines

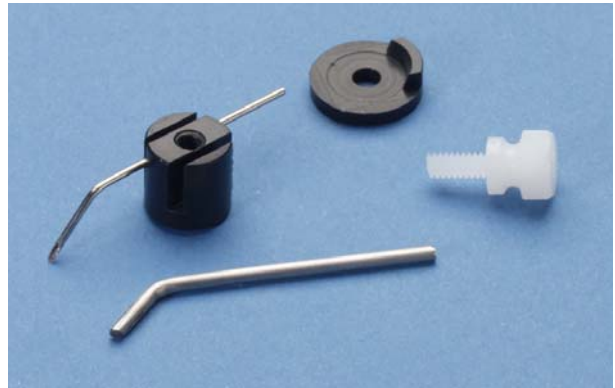
17. Attach input and output perfusion lines to the APICAL and BASOLATERAL CHAMBERS. The *upper perfusion ports* are connected to the APICAL CHAMBER and the *lower perfusion ports* connect to the basolateral chamber.

NOTES: Pre-fill perfusion lines before making connections. Use care to not disturb the seal between the chambers and sample when making connections. Perfusate is delivered to the **RC-50/E** through the supplied **PE-90** tubing. **PE-90** tubing fits neatly inside **PE-160** tubing. This allows the **RC-50/E** to be used with in-line heaters such as Warner's **SH-27B**.

18. If configured for *closed bath* use, connect input and output perfusion lines to both the BASOLATERAL and APICAL CHAMBERS. The direction of fluid flow within the chambers is not important and is left to the user's discretion.
19. If configured for *open bath* use, connect input and output perfusion lines to the BASOLATERAL CHAMBER and an input perfusion line to the APICAL CHAMBER. The direction of fluid flow within the BASOLATERAL CHAMBER remains arbitrary.

NOTE: Connect the apical input perfusion line to the perfusion port furthest away from the bath cutout.

20. If configuring for *open bath* use, place a SUCTION TUBE ASSEMBLY (shown to the right) into the output side of the bath. Connect the suction tube to an aspirator.



Mount the Chamber into the Platform

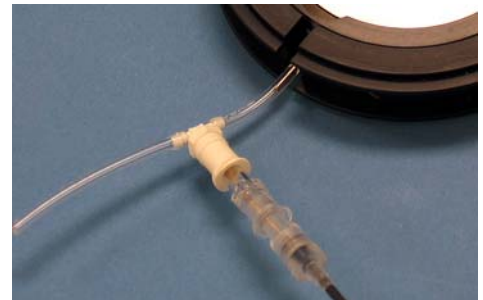
21. Place the assembled Ussing chamber into the platform and tighten the pressure plate onto the place using the pressure plate wrench. A completed open bath assembly is shown to the right. Note the position of the suction tube assembly.



Attaching electrodes (RC-50E only)

Current and voltage electrodes must be assembled and filled prior to insertion into the RC-50E. Assembly instructions for both the current and voltage electrodes can be found in the Appendix.

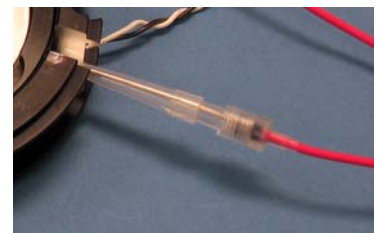
22. Attach one CURRENT ELECTRODE T-FITTING to the outflow perfusion line for the chamber's basolateral side, and the other CURRENT ELECTRODE T-FITTING to the outflow perfusion line for the apical side.
23. Pre-fill the T-fitting with buffer and insert the assembled CURRENT ELECTRODES into the T-fitting. Perform this step for both the basolateral and apical T-fittings.



NOTE: Slightly overfilling the T-fittings will force any trapped bubbles into the solution's flow-to-waste when the electrode is inserted. This will insure good electrical contact between the electrode and the chamber's perfusing solutions.

24. Insert the VOLTAGE ELECTRODES into their basolateral and apical receptacles on the **RC-50E** chamber body.

NOTE: Current and voltage electrodes come in pairs; a red set for the basolateral side and a black set for the apical side.



MOUNTING ONTO THE MICROSCOPE

The **RC-50/E** chamber can be mounted directly onto a microscope stage if the stage is both flat and has a cutout which fits the platform. In most cases, however, the stage cutout differs from the platform geometry necessitating the use of a stage adapter. Warner Instruments stocks stage adapters for most popular microscopes (see Appendix A) and we will custom manufacture adapters for special applications. Contact our Sales Department for details.

FLUID CONTROL

The selection of solution sources can be of either manual or automatic design and is left to the user. However, Warner Instruments manufactures several perfusion control systems (such as the valve-driven **VC-8** and **VC-8M Control Systems**) which can be used with this application.

Solution delivery can be of a pumped or gravity feed design. In general, a pumped approach will best serve solution delivery and removal for the apical and basolateral chambers in the *closed bath* configuration.

The use of a pump is also recommended for solution delivery and removal for the basolateral chamber in the *open bath* configuration. Solution delivery to the apical chamber can either be pumped or gravity fed since the perfusate is removed by aspiration.

PLATFORM HEATING

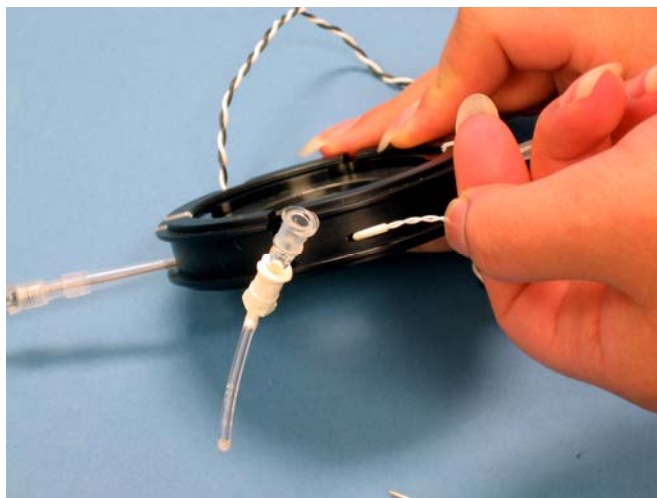
Monitoring the heat

Heat is transferred to the platform from a pair of 20 Ω power resistors, mounted on the underside of the platform. Platforms are supplied with a thermistor assembly. The temperature of the platform is monitored by measuring the platform thermistor resistance and adjusting the voltage to the heaters.

Automatic heat control can be achieved by using either a Warner **TC-324B** or **TC-344B** Temperature Controller (single or dual channel models, respectively). These devices allow the platform or a solution thermistor to be selected as the control sensor. The desired temperature is set and automatically maintained at less than 1°C deviation.

Feedback control by the **TC-324B** or **TC-344B** is communicated from the chamber to the controller by use of a **CC-28** thermistor cable. In particular, the white bead thermistor is inserted into the size matched hole in the side of the **RC-50/E** base. See the image to the right for hole location.

NOTE: You can use a small amount of mineral oil to facilitate a tight thermal couple between the thermistor and the chamber.

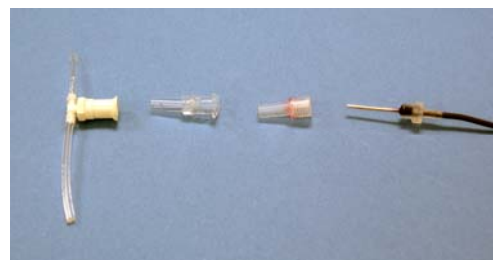


APPENDIX

Assembly of current electrodes

Current delivery electrodes mount to the chamber via a T-fitting placed in-line with the perfusion outlet lines on both apical and basolateral chamber sides.

The current electrode is comprised of the components shown to the right. These components, from left to right, are termed the T-FITTING, the LUER FITTING, the BRIDGE FITTING ADAPTER, and the ELECTRODE TERMINUS.



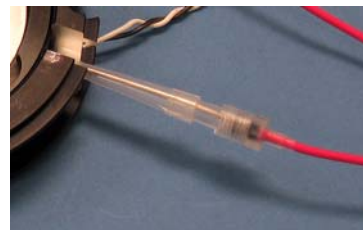
1. Begin by screwing the ELECTRODE TERMINUS into the BRIDGE FITTING ADAPTER. Trim the electrode wire so that it extends no more than 1 mm from the end of the BRIDGE FITTING ADAPTER. Separate the two components.
2. Chloride the Ag^+ wire in the ELECTRODE TERMINUS.
3. Prepare for making salt bridges by melting 3% agar into 3 M KCl.
4. Place the LUER FITTING onto a 1 cc syringe and carefully draw the hot agar into the LUER FITTING until the *fill region* is full. Allow to cool.
5. Insert the LUER FITTING into the BRIDGE FITTING ADAPTER. Fill the assembly to the top with 3 M KCl.
6. Carefully screw the ELECTRODE TERMINUS into the BRIDGE FITTING ADAPTER to complete the assembly. The current electrode can now be attached to its associated T-FITTING.



Assembly of voltage electrodes

Voltage sensing electrodes mount to the chamber via ports positioned on both the apical and basolateral sides of the chamber.

1. Begin by screwing the ELECTRODE TERMINUS into the PIPETTE TIP SOLUTION RESERVOIR. Trim the electrode wire so that it extends no more than 1 cm into the SOLUTION RESERVOIR. Separate the two components.
2. Chloride the Ag^+ wire in the ELECTRODE TERMINUS.
3. Prepare for making salt bridges by melting 3% agar into 3 M KCl.
4. Place the PIPETTE TIP SOLUTION RESERVOIR onto a 1 cc syringe and carefully draw hot agar into the PIPETTE TIP SOLUTION RESERVOIR until the pipette tip is approximately $\frac{1}{2}$ full. Allow to cool.
5. Fill the top of the PIPETTE TIP SOLUTION RESERVOIR with 3 M KCl.
6. Carefully screw the ELECTRODE TERMINUS into the SOLUTION RESERVOIR to complete the assembly. The voltage electrode can now be attached to its associated port on the chamber.



Chloriding silver wire electrodes

General Precautions

1. To avoid contamination do not touch electrode with bare fingers.
2. Hold the electrode with tweezers at the base of the silver wire to prevent breakage when working with bare disks and pellets,. Avoid bending the wire more than necessary.

3. Electrodes should not be mounted in direct contact with metals to prevent undesirable chemical side-reactions.

Chloriding

Unused Ag/AgCl wire should be clean before chloriding. This is easily achieved by wiping with alcohol.

Chloriding wire is achieved by making it positive, relative to a solution containing NaCl (0.9%) or KCl (3 M) and passing a current at a rate of 1 mA/cm² of surface area for about a minute, or until adequately plated (a 2 cm length of the 0.25 mm wire used in the holders would require 0.15 mA). The color of a well plated wire should be light gray. Occasionally reversing the polarity for several seconds while plating tends to yield a more stable electrode.

An alternate method of chloriding is to immerse the wire in Clorox until a light gray color is observed (typically 15 seconds to several minutes).

Regardless of the technique used, rinse the electrode after plating and store or use.

Electrode Conditioning

To minimize DC offset and the noise of a pair of disk or pellet electrodes, immerse the pair in a selected electrolyte for 24 hours. This procedure allows the interstitial solution phase to come into equilibrium with the outside electrolyte.

NOTE: Electrodes should be soaked with their wires shorted to each other. Avoid touching or contaminating the bare metal surface, otherwise the offset will increase.

Another technique for lowering offset and polarization of an electrode pair just before use is to apply an AC voltage while they are immersed in a saline solution. The current should be a few mA and should be applied for no longer than 2 minutes. The applied AC frequency should be between 50 and 400 Hz.

NOTE: While results with different electrolytes are not predictable experimentation usually will not damage the electrodes unless corrosive chemicals are used.

Cleaning

After most applications, rinsing the electrodes with distilled water or isopropyl alcohol will suffice. Should the electrode surface be especially dirty or corroded, a new surface can be exposed by gently sanding off the pellet with fine sandpaper or abrading with a pumice cleanser (do not use emery cloth).

Mounted electrodes can be cleaned by scrubbing with a brush and detergent water, follow by rinsing thoroughly with running water, drying and storing as recommended. Acetone or other strong solvents should not be used.

Sterilization

When sterilization is required, only gas or liquid agents should be employed. Disinfectants containing mercury, phenols, bromine, iodine, zinc, tin or organometallic compounds should be avoided. Do not autoclave mounted electrodes as most epoxies and cable insulations cannot withstand steam sterilization.

Darkening

Silver chloride is light-sensitive. Exposure to light will darken the electrode surface. This will not impair the electrode's performance, however, since the large interstitial Ag/AgCl matrix is not reached by light.

Should the dark surface be undesirable, the coating can be removed by gently sanding with a fine sandpaper or abrading with a pumice cleanser (do not use emery cloth), follow by rinsing thoroughly with running water and drying. Acetone or other strong solvents should not be used.

Temperature Limit

Silver-silver chloride electrodes can withstand temperatures up to 2000 °C. Lower temperature limits should be imposed if the electrodes are used with adhesive or encapsulating materials.

Storage

Bare electrode pellets and disks should be handled with care. Do not store electrodes in contact with any metals, especially active metals as iron or aluminum. Also, substances that could affect the electrochemical characteristics of the materials used should be avoided. Bare electrodes should be stored in a clean, dry and dark container (plastic or glass).

Maintenance

Perfusion port cleaning

The PERFUSION PORT CLEANOUT TOOL (shown on page 4) can be used to clear the perfusion ports if they become clogged with either protein or vacuum grease. The tool may need to be applied from *both ends* of the port since the flow channel has two turns in it. Care must be taken when cleaning these turns to insure that the small plugs placed during fabrication are not removed. If the plugs are accidentally removed, they can be readily re-sealed using RTV (Corning 732).

Note: The RC-50/E perfusion ports should be cleaned immediately after each use.

General cleaning

Cleaning of all RC-50/E components can be performed using a dilute detergent solution. Alternatively, Warner instruments has developed a trisodium phosphate (TSP) wash protocol which is effective in cleaning plastic parts. Contact our Technical Support staff or download the protocol in PDF format from our website.

NOTE: Do not use alcohol, ether or other solvents on plastic parts. Solvents may be used on the anodized surfaces of the platforms. Aluminum chamber parts may also be autoclaved.

Warner Stage Adapters (see www.warneronline.com for an updated listing)

Microscope Manufacturer	Warner Instrument Stage Adapter Model No.
Nikon Diaphot, TE 200 & TE 300	SA-30 NIK
Nikon TMS with 8 x 12 cm stage cutout	SA-30 TMS8
Nikon TMS with 9 x 13 cm stage cutout	SA-30 TMS9
Olympus IMT (older model)	SA-30 OLY
Olympus IMT-2, IX, & BX50WI	SA-30 OLY/2
Zeiss Axiovert, Leica (Lietz) DMIRB & DMIL	SA-30 LZ
Leica (Lietz) DMIRB/E with 3-plate	SA-30 L3P
Zeiss K stage	SA-30 KZ
Prior and Ludl motorized stages on inverted stages	SA-30 PLI

NOTE: Warner Instrument **Series P** platforms are designed to fit the Zeiss 76x26 microscope slide frame (#471719) without a stage adapter. Heater platforms will require an insulating material between the platform and frame.

Comments

- 1) Silicone vacuum grease (also called stopcock grease) is available from Warner Instruments. (Warner model 111)
- 2) Best temperature regulation is achieved by preheating the perfusion solution with an in-line heater (Warner model SH-27B or SF-28) in addition to warming the chamber.